STANDARD OPERATING PROCEDURE

BD Sorter Monthly/Post-Contamination Cleaning

Revision No:	1.1	**Revision Date:**	09/29/2025
Revision By:	MB	**Reviewed By:**	MB

1. Purpose:

1.1. To ensure the BD Cell Sorter remains sterile and free of contamination through routine monthly cleaning or post-contamination cleaning procedures.
1.2. This SOP applies to all personnel responsible for operating and maintaining the BD Cell Sorter(s) in the Flow Cytometry Core Facility.
1.3. Operators are responsible for performing the cleaning protocol and documenting sterility results.

2. Health and Safety:

2.1. Observe standard bloodborne pathogen precautions when working with any biological samples.
2.2. Observe standard laboratory techniques and wear appropriate personal protective equipment for handling chemicals and biologics. Refer to the appropriate Safety Data Sheet for specific requirements.
2.3. Caution should be taken when using hydrogen peroxide (H_2O_2). It must be diluted from 30% stock to a working concentration of 3% (150 ml 30% H_2O_2:1.5 L H_2O V_t; 450 ml 10% H_2O_2:1.5 L H_2O V_t), as it can react with the bleach at higher concentrations.

3. Materials and Equipment:

3.1. 10% Bleach
3.2. 70% Ethanol (EtOH)
3.3. 3% Hydrogen Peroxide (H_2O_2)
3.4. Millipore water
3.5. Sterile 1x PBS
3.6. Autoclaved sheath tank components
3.7. EtOH cleaning tank
3.8. Waste tank

3.9. Sheath filter
3.10. LB tubes (1ml LB in 5ml FACS tube)
3.11. Incubator (37°C)
3.12. Sterility log

4. Procedure:

4.1. Sterility Sampling (Daily)
4.1.1. After fluidics startup and QC, run 10% bleach followed by sterile H_2O.
4.1.2. Annotate an LB tube with: Date, Initials, Instrument name.
4.1.3. Collect stream for 10 seconds into the tube.
4.1.4. Incubate at 37°C.
4.1.5. Check for cloudiness/contamination growth each morning and afternoon for 7 days.
4.1.6. Record results in the sterility log.

Example of sterility log used to track contamination and cleaning with day of the month in the column and days 0-7 in rows.

4.2. Sheath Filter Management
4.2.1. Remove the sheath filter and connect the sheath lines directly. If filter is new: Label with date and initials, Place one mark. For each monthly clean, add a mark. If filter has 3 marks or contamination is detected, discard. If reusable, set aside until H_2O_2 step.

4.3. Cleaning Steps
4.3.1. Bleach Flush
Empty and rinse the EtOH tank with Millipore water then fill with 1.5 L of 10% bleach. Attach sheath and air lines **(without sheath filter)** and run *fluidics startup* from the Cytometer Menu.
4.3.2. Ethanol Flush

Empty bleach from EtOH tank with rinse with Millipore water then fill with 1.5 L of 70% EtOH. Empty and rinse waste tank; leave empty and reattached to waste line. Attach sheath and air lines **(without sheath filter)** and run *fluidics startup* from the Cytometer Menu.

4.3.3. Hydrogen Peroxide Flush

Empty EtOH tank with rinse with Millipore water then fill with 1.5 liter of 3% hydrogen peroxide (150mls 30% H_2O_2:1.35L H_2O). (Very important to dilute the H_20_2, it can react with the bleach at higher concentrations). **Install a clean sheath filter** and attach sheath and air lines and run *fluidics startup* from the Cytometer Menu.

4.3.4 Water Flush

Empty H_2O_2 and fill with 1.5 liter of Millipore water attach sheath and air lines (with sheath filter) and run *fluidics startup* from the Cytometer Menu.

4.4 Sheath Tank Preparation

4.4.4 Assemble fresh sheath tank using autoclaved parts. Label with clean date and initials. Place assembled tank on scale and fill with ~16 kg sterile 1x PBS.

4.4.5 Disconnect lines from EtOH tank and connect to sheath tank.

4.4.6 Empty and rinse waste tank fill with 500 mL undiluted bleach and reconnect to waste line. Run *fluidics startup* from the Cytometer Menu.

4.4.7 Proceed with daily instrument cleaning, QC, and sterility monitoring procedure.

4.5 Disassemble old sheath tank

4.5.4 Remove fittings, sheath sensor and any plumber's tape.

4.5.5 Place sheath sensor in a graduated cylinder filled with 70% EtOH up to the top of the probe to soak.

4.5.6 Wash tank and quick connect fittings with antibacterial soap, rinse with Millipore water.

4.5.7 Place fittings in an autoclave bag and label with lab, date and contents.

4.5.8 Send tank and fittings to glassware to be autoclaved.